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Soil Electrical Conductivity and Water Content Affect Nitrous Oxide and Carbon Dioxide Emissions in Intensively Managed Soils

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ABSTRACT

Accumulation of soluble salts resulting from fertilizer N may affect microbial production of N_2O and CO_2 in soils. This study was conducted to determine the effects of electrical conductivity (EC) and water content on N_2O and CO_2 production in five soils under intensive cropping. Surface soils from maize fields were washed, repacked and brought to 60% or 90% water-filled pore space (WFPS). Salt mixtures were added to achieve an initial *in situ* soil EC of 0.5, 1.0, 1.5 and 2.0 dS m^{-1}. The soil cores were incubated at 25°C for 10 d. Average CO_2 production decreased with increasing EC at both soil water contents, indicating a general reduction in microbial respiration with increasing EC. Average cumulative N_2O production at 60% WFPS decreased from 2.0 mg N_2O-N m^{-2} at an initial EC of 0.5 dS m^{-1} to 0.86 mg N_2O-N m^{-2} at 2.0 dS m^{-1}. At 90% WFPS, N_2O production was 2 to 40 times greater than that at 60% WFPS and maximum N_2O losses occurred at the highest EC level of 2.0 dS m^{-1}. Differences in the magnitude of gas emissions at varying WFPS were due to available substrate N and the predominance of nitrification under aerobic conditions (60% WFPS) and denitrification when oxygen was limited (90% WFPS). Differences in gas emissions at varying soil EC may be due to changes in mechanisms of adjustment to salt stress and ion toxicities by microbial communities. Direct effects of EC on microbial respiration and N_2O emissions need to be accounted for in ecosystems models for predicting soil greenhouse gas emissions.

Application of high amounts of fertilizers and poor quality irrigation water may cause an accumulation of soluble salts in agricultural soils. Soluble salt concentrations (e.g., Ca^{2+}, Mg^{2+}, K^+, Na^+, H^+, NO_3^-, SO_4^{2-}, Cl^-, HCO_3^-, CO_3^{2-}, OH^-) in soils are generally measured in terms of electrical conductivity (EC) (Rhoades, 1993, Smith and Doran, 1996). Most soils are considered slightly saline if the EC of a saturated paste extract exceeds 2 dS m^{-1} (Smith and Doran, 1996). Microbial growth, nitrogen transformations, and decomposition of organic matter are affected by high concentrations of dissolved cations and anions in soils (Mendum et al., 1999; Avrahami et al., 2002; Killham, 1985; Irshad et al., 2005; Laura, 1974; Frankenberger and Bingham, 1982).

Microorganisms vary widely in their tolerance to salt stress, but bacterial processes such as nitrification and denitrification can be greatly affected by soil EC at levels that are well below the commonly used salinity thresholds (Smith and Doran, 1996). In the absence of soil salinity, changes in soil nutrient levels influence soil EC through differences in the type and number of cations and anions held by the soil particles. Soil water content influences soil EC through the concentration of dissolved ions in the soil. When soil water content is high, dissolved ions (solutes) are diluted; when soil water content is low, dissolved ions are concentrated. This relationship also affects water and substrate supply to microbial cells. To adapt to increased soluble salts at low soil water content, microorganisms create internal solute concentrations either by producing compatible organic solutes or by taking up ions from the extracellular solution (Csonka, 1989). Since water availability is an important factor influencing microbial cells, microbial processes such as nitrification, denitrification and soil respiration are greatly affected.

Application of fertilizer N often leads to transient, localized, and high concentrations of soil solutes. High salt concentrations may cause an inhibition of nitrification after the addition of fertilizer N. Significant increases in N_2O emissions may particularly occur when fertilizer N is applied in excess of crop N demand. More generally, fertilizer effects on N_2O emission can vary widely, depending on factors such as soil type, the amounts and forms of N applied, or N application methods (Granli and Bockman, 1994; Eichner, 1990). Application of 56 to 224 kg N ha^{-1} as NH_4NO_3 to barley plots in northeastern Colorado resulted in total N_2O emissions that averaged 0.5% of the fertilizer added (Mosier et al., 1982). In another study, the gaseous N loss associated with the addition of $Ca(NO_3)_2$ or urea (120 kg ha^{-1} N) to a maize field over an 85 d period of sampling was 0.33 and 2.5% of the N applied, respectively (Duxbury and McConnaughey, 1986). Likewise, Breitenbeck et al. (1980) observed that emissions of N_2O-N during a period of 96 d from soil treated with NH_4^+ fertilizers (125–250 kg N ha^{-1}) were 0.11 to 0.14% of the N applied as compared to only 0.01 to 0.4% for $Ca(NO_3)_2$. McSwiney and Robertson (2005) reported that 7% of fertilizer N was lost as N_2O at an N application rate of 134 kg N ha^{-1} in a continuous maize cropping system managed at relatively low yield levels. Total N_2O emission increased for N applications greater than 134 kg N ha^{-1}, but decreased to 2 to 4% of the fertilizer N applied in relative terms. The authors speculated that the drop of N_2O flux was due to a change in the microbial processes that produced N_2O and controlled N availability.

Many field studies suggest that significant soil N_2O fluxes are most likely to occur when water-filled pore

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Abbreviations: BD, Bulk density; EC, Soil electrical conductivity; PAS, Infrared photoacoustic spectroscopy; WFPS, Water-filled pore space.

space (WFPS) exceeds 60%, soil nitrate concentration is >5 mg NO_3–N kg^{-1} dry soil, and soil temperature is >5°C (Abbasi and Adams, 2000; Dobbie and Smith, 2003; Kessavalou et al., 1998; Qian et al., 1997; Sehy et al., 2003; Simojoki and Jaakkola, 2000). At constant soil water content and temperature, rates of soil N_2O and CO_2 production and emission are primarily associated with substrate supply for microbial respiration, nitrification, denitrification, and other external factors that may influence the microbial community. Solute salt concentration, measured as EC, is one of those external factors, but its direct influence on soil greenhouse gas emissions is little understood.

Linear relationships between EC and soil nitrate content in nonsaline soils have been observed in several studies. Smith and Doran (1996) found high positive correlations between EC and NO_3–N for fall and summer measurements in a winter wheat–dry pea rotation. Patriquin et al. (1993) also observed a linear relationship between EC and soil nitrate content in shallow groundwater of a maize field in Canada. The presence of NO_3^- is one prerequisite for N_2O production. Since soil NO_3–N content tends to be positively correlated with N_2O emissions (Mosier et al., 1983), we may assume that a close relationship also exists between soil EC and N_2O emissions.

Although there are many studies of specific ion and soil water effects on microbial processes, the range of soil EC (2.5 to 20 dS m^{-1}) used was mostly outside EC levels found in agricultural soils (Wijler and Delwiche, 1954; Irshad et al., 2005; Heilman, 1975; Dinesh et al., 1995; Darrah et al., 1987; Frankenberger and Bingham, 1982; McClung and Frankenberger, 1985). No studies that we are aware of have investigated the effect of soil EC < 2.0 dS m^{-1} and soil water content at relatively low levels of soil NO_3–N content on greenhouse gas production in soil. One reason may be that it is difficult to separate the role of soil EC from soil NO_3–N content, since these factors are often interdependent. Amos et al. (2005) conducted field measurements of N_2O and CO_2 fluxes in an irrigated maize system. Based on different sampling events during the growing season, they reported a positive correlation between N_2O emission and soil EC at WFPS of 54 to 68%. However, in their case, high N_2O emissions coincided with high fertilizer input, i.e., the increased EC resulted from fertilizer applications and the high N_2O fluxes may have been caused by high soil NO_3–N levels, not necessarily due to a direct effect of EC on microbial activities.

Our hypothesis was that soil N_2O emissions are increased at high soil EC levels by shifting microbial N transformations toward pathways that favor N_2O production and that this occurs in the presence of relatively low initial soil nitrate content. A laboratory experiment was conducted to (i) quantify how soil EC and water content affect the emissions of N_2O and CO_2 in agricultural soils and (ii) determine the ranges of initial soil EC that inhibit or increase N_2O production at low levels of nitrate concentration in the soil.

MATERIALS AND METHODS

Soils

Five soils representing different intensive maize-based cropping systems were used in this study (Tables 1 and 2). Two fresh samples of Kennebec silt loam soils (fine-silty, mixed, superactive, mesic Cumulic Hapludolls) (0 to 0.3 m depth) were collected from the Ecological Intensification of Irrigated Maize-based Cropping Systems Experiment located at the University of Nebraska in Lincoln, NE (40°82′ N; 96°65′ W; elev. 357 m). This experiment was established in 1999 to identify efficient crop management practices for achieving yields that approach yield potential levels and to determine resource-use efficiencies and greenhouse gas fluxes in intensively managed maize and soybean systems (Amos et al., 2005). Samples were collected from two contrasting cropping systems (Table 2): (i) Lin-CC: continuous maize with intensive management (high plant density, intensive NPK management) and (ii) Lin-CS: maize–soybean rotation with recommended best management (normal plant density, recommended NPK management). In each treatment replicate plot, three soil cores (33 mm i.d.) were taken between maize rows after harvest in 2005. The twelve soil cores were thoroughly mixed to form one composite sample per treatment.

Another two fresh soil samples of a Tomek silty clay loam (fine, smectitic, mesic, Pachic Argialbolls) were collected in 2005 from two no-till systems located at the University of Nebraska Agricultural Research and Development Center near Mead, NE (41°15′ N; 96°48′ W; elev. 366 m). Both sites represent intensively managed, irrigated no-till cropping (for about 15 yr). Since 2001, the Mead-CC soil was managed as a continuous maize system, whereas the Mead-CS soil was under a maize–soybean rotation. Input management followed established recommended management practices and yields at both sites were generally high (Verma et al., 2005). Fertilizer N applied was 200 to 230 kg N ha^{-1} at the Mead-CC site and 160 to 200 kg N ha^{-1} at the Mead-CS site. Within each site, twenty soil cores (33 mm i.d.; 0 to 0.3 m depth) were taken between maize rows in 20 by 20 m detailed measurement areas after the harvest in 2005. These soil cores were thoroughly mixed to form one composite sample per site.

Table 1. Characteristics of the soil samples used for the laboratory experiment.

Soil	Classification	Soil texture						Mineral N‡	
		Sand	Silt	Clay	$EC_{1:1}$†	Organic C	pH†	NO_3–N	NH_4–N
		g kg^{-1}			dS m^{-1}	g kg^{-1}		μg g^{-1}	
Lin-CS	Fine-silty, mixed, superactive, mesic cumulic Hapludolls	50	580	370	0.33	15.9	6.48	1.03	10.9
Lin-CC	Fine-silty, mixed, superactive, mesic cumulic Hapludolls	50	580	370	0.20	15.9	6.38	1.28	10.3
Mead-CS	Fine, smectitic, mesic Pachic Argialbolls	110	540	350	0.28	18.0	6.49	0.69	16.1
Mead-CC	Fine, smectitic, mesic Pachic Argialbolls	120	580	300	0.28	18.0	6.42	1.76	16.6
Man-CC	Fine-loamy, mixed, superactive, Aquic Hapludolls	310	330	360	0.31	29.2	6.41	0.26	26.9

† Soil $EC_{1:1}$ = soil electrical conductivity measured in 1:1 soil:water suspension after washing with deionized water.
‡ Soil mineral N = soil N measured in 2 *M* KCl extracts after washing with deionized water.

Table 2. Crop management practices and crop productivity levels of the study soils.

	Lin-CS	Lin-CC	Mead-CS	Mead-CC	Man-CC
Tillage	**Fall plow**	**Fall plow**	**No-till**	**No-till**	**Fall plow**
Irrigation	**Yes**	**Yes**	**Yes**	**Yes**	**No**
Plant population maize, pl. m^{-2}	**7–7.5**	**9–10**	**8**	**8**	**9**
N application, maize, kg N ha^{-1}	**120–140**	**250–300**	**170–200**	**200–230**	**400–500**
No. of N applications per crop	**2**	**4–5**	**3–4**	**3–4**	**3**
P application, maize, kg P ha^{-1}	**0**	**45**	**10–20**	**10–20**	**10–20**
K application, maize, kg K ha^{-1}	**0**	**85**	**0**	**0**	**30**
Maize grain yields, Mg ha^{-1}	**14–16**	**15–17**	**12–13**	**13–14**	**16–20**

The Floyd–Webster loam (Man-CC) soil (fine-loamy, mixed, superactive, Aquic Hapludolls) was collected from the farm of Francis Childs, a frequent winner of maize yield contests, located at Manchester, Iowa (42°47′ N; 91°45′ W; elev. 302 m). The field is under rainfed continuous maize cropping for >30 yr. Crop management practices include application of 400 to 500 kg N ha^{-1} and high plant density (Murrell and Childs, 2000). Soil samples were collected from three 7 by 12 m strips of a high-yield demonstration plot managed by Mr. Childs. Twelve soil cores (33 mm i.d.; 0 to 0.3 m depth) were taken between maize rows at harvest stage of maize in 2002 and thoroughly mixed to form one composite sample.

Laboratory Incubations

Two L of deionized water was added to 1.2 kg of each soil and the soils were placed on a shaker for 30 min to remove soluble ions. After shaking, the soil suspension was allowed to stand for 1 to 2 h. Standing water was removed and the remaining soil paste was tested for soil EC using a pencil conductivity probe (Hanna Instruments Dist WP, Woonsocket, RI). Soil washing was repeated for several times until soil electrical conductivity measured in 1:1 soil/water suspension ($EC_{1:1}$) reached ≤ 0.5 dS m^{-1}. The soils were air-dried for at least 3 d to a volumetric water content (θ_V) of <5%. After drying, the soils were crushed, passed through a 2-mm sieve, and stored in airtight glass containers before soil incubation. Ten grams of soil (dry weight basis) were weighed and 80 mL of 2 *M* KCl was added. The suspension was shaken for 30 min and filtered through a Whatman no. 42 filter paper. Soil NO_3–N contents in soil extracts were measured using the Cd-reduction method (Mulvaney, 1996), whereas soil NH_4–N contents were determined by the salicylate method (Kempers and Zweers, 1986). Both soil NO_3–N and NH_4–N contents were expressed in mg N per kg dry soil, and the results are summarized in Table 1. A 50-g sample of soil was analyzed for soil texture and soil organic C (Table 1).

Soil cores for incubation were prepared as follows. Fifty grams of each soil (dry weight basis) were weighed into a 100-mL beaker and compacted to a bulk density of between 1.09 and 1.14 Mg m^{-3}. Each of the soils was treated with KCl, $CaCl_2$ and Na_2SO_4 salt mixtures dissolved in deionized water at rates that produced electrical conductivities of the bulk soil of approximately 0.5, 1.0, 1.5, and 2.0 dS m^{-1} and water-filled pore space of 60 and 90%. All salt treatments had an ionic ratio of Cl^-: SO_4^{2-}: K^+: Ca^{2+}: Na^+ = 3: 2: 1.5: 1: 1. The EC values were achieved by carefully pipetting 1 to 8 mL of salt solution per 50 g of soil on a dry weight equivalent basis into a test tube. The salt solution was brought to a final volume by adding deionized water that produced a water-filled pore space of 60 and 90%. The salt solution was shaken using a vortex mixer and gently poured into the soil. Before soil incubation, each soil was tested to estimate the amounts of salt and volume of deionized water that produced 0.5, 1.0, 1.5, and 2.0 dS m^{-1} and WFPS of 60 and 90%. Water contents of the five air-dried soils were determined by drying soils for at least 2 d at 105°C. The volume of water added to salt solution per soil core was adjusted using the equation:

$$\theta_g \; at \; \%WFPS = \frac{(WFPS) \times \left(1 - \dfrac{BD}{2.65}\right)}{BD} \qquad [1]$$

where θ_g at % WFPS is gravimetric soil water content at 60 or 90% WFPS (%), WFPS is the fraction of pore space filled with water (%), BD is the bulk density of the soil core, and 2.65 is the soil particle density (Mg m^{-3}). The salt concentrations used for each soil ranged from 0.009 to 0.477 *M* KCl, $CaCl_2$, and Na_2SO_4 at 60% WFPS and 0.001 to 0.144 *M* KCl, $CaCl_2$, and Na_2SO_4 at 90% WFPS. We chose K^+, Ca^{2+}, Na^+, SO_4^{2-}, and Cl^- ions as electrolytes because these are common ions in many cultivated soils and in irrigation water. Soil water contents of 60 and 90% WFPS were chosen because these levels represent previously reported thresholds for maximum microbial nitrification and denitrification, respectively (Linn and Doran, 1984). The soil cores were placed individually inside a 2-L glass jar with a screw-cap lid in which a septum was fitted for gas sampling. The jars were incubated at 25°C for 10 d. Each treatment was replicated three times in a randomized complete block design. For the 90% WFPS treatment, another duplicate set of 50 g soil cores was repacked and treated with salt solution for measurement of soil redox potential.

Gas and Soil Analyses

During the 10-d incubation, gas samples of the headspace of all jars were analyzed daily for N_2O and CO_2 concentrations using a Model 1312 infrared photoacoustic spectroscopy (PAS) gas analyzer (Innova Air Tech Instruments, Ballerup, DK). All measurements were done using a closed-loop system. The jar headspace was sampled using a flow-through lid consisting of rubber septa on jar lids and a 5-cm stainless infusion needle (gauge # 19) connected to the inlet and outlet ports of the PAS gas analyzer. Since the needles used for gas analyses were impervious to air and water, this setup permitted a closed-loop system in which an equivalent amount of air was returned to the jar through the outlet port of the PAS gas analyzer every time a gas sample was drawn through the inlet port. Immediately after the end of each gas analysis, the jars were flushed with atmospheric air for 3 min before the jars were resealed with silicon sealant and returned to the incubator. Water content of soil cores was maintained by weighing and adding necessary deionized water to incubating soil cores. Control samples were included to correct for small amounts of N_2O and CO_2 contained in jars without soils. The control jars contained atmospheric N_2O of 0.262 to 0.382 mg L^{-1} and ambient CO_2 of 482 to 516 mg L^{-1}. There was no system backpressure every time the PAS gas analyzer drew air from the headspace, suggesting that the 1 d incubation did not cause over-pressurization of the jar. The PAS instrument was set at 1-min intervals and measurements of N_2O and CO_2 concentrations in each jar were conducted in triplicates. The production of N_2O and CO_2 was estimated by

subtracting the amount of the N_2O and CO_2 in control jars from the amount of N_2O and CO_2 evolved from soil cores. The results were averaged and expressed in terms of gas concentration. The N_2O and CO_2 emission rates were calculated as follows:

$$F = \frac{\Delta C}{\Delta t} \times \frac{V}{A} \times \rho \times \alpha \quad [2]$$

where F is the gas production rate for N_2O (g N_2O-N m^{-2} d^{-1}) or CO_2 (g CO_2–C m^{-2} d^{-1}), $\Delta C/\Delta t$ denotes the change in gas concentration in the headspace (10^{-6} L L^{-1} d^{-1}), V is the headspace volume of the jar (L), A is the surface area of soil core (m^2), ρ is the density of gas at 20°C and 0.101 M Pa (1 mol per 24.04 L), and α is a conversion coefficient (28/44 for N_2O and 12/44 for CO_2).

Redox potentials of the soil cores were measured after 1-, 3-, 7-, and 10-d incubation periods using an Eh meter (Orion 525A+ meter, Thermo Electron, USA). A platinum electrode was placed at the middle of the soil core for at least 30 min or until a relatively stable readout was obtained. The Eh measurements were expressed in mV and are presented in Table 3.

Post Incubation Analyses

At the end of the 10-d incubation, the soil cores inside the jars were removed and mixed. A 10-g fresh weight soil sample was shaken with 10 mL of deionized water for 30 min. After shaking, soil EC was measured using a pencil conductivity probe (Hanna Instruments Dist WP, Woonsocket, RI). Another 10-g fresh weight soil was shaken with 2 M KCl for 30 min and was analyzed for soil NO_3–N and NH_4–N concentration using the methods discussed previously. Soil N contents were expressed in mg N per kg dry soil.

Statistical Analyses

The five soils, four levels of soil EC and two water contents were replicated three times in a $5 \times 4 \times 2 \times 3$ factorial design. Statistical analyses were performed using the General Linear Model procedure (proc GLM) in SAS (SAS, 1999). An analysis of variance (ANOVA) was performed to determine significant differences of N_2O and CO_2 production at different levels of soil EC and water content among all soils and within each soil. Least significant difference values (LSD) were calculated at $P < 0.05$, when the effects of soil EC and WFPS on N_2O and CO_2 production were found to be significant. All analyses were performed using proc GLM (SAS, 1999).

RESULTS

Mineral Soil Nitrogen Content and Soil Electrical Conductivity

All soils had low levels of NO_3–N before salt additions. After a series of soil washings, initial mineral soil content ranged from 0.26 to 1.76 mg NO_3–N kg^{-1} and 10.3 to 26.9 mg NH_4–N kg^{-1} (Table 1). Incubation at 60% WFPS resulted in an increase in NO_3–N compared to initial levels, but a negative relationship between final NO_3–N content and soil EC (Table 3). Final NO_3–N was lowest at EC levels greater than 1.5 dS m^{-1}, particularly in the Mead and Manchester soils ($P = 0.006$). At 90% WFPS, final NO_3–N levels in all soils were very low and not affected by EC, indicating nearly complete loss of nitrate by denitrification (Table 3). On average, soil EC after 10 d incubation at 60 or 90% WFPS decreased only slightly (<10 to 15%) from the initial EC levels that had been established by salt addition (Table 4).

Soil NH_4–N content decreased at both water contents and in all EC treatments from pre-incubation levels in all soils except for the Man-CC soil. This soil had a high organic matter content (29 g C kg^{-1}) and is known to have exceptionally high rates of microbial activities (R. Drijber, unpublished data). The levels of salt treatment significantly ($P = 0.024$ to 0.004) affected the final NH_4–N content of most soils. The largest soil NH_4–N content was generally observed during incubation at 60% WFPS and at a soil EC of 2.0 dS m^{-1}, whereas NH_4–N content tended to be lowest in soils with low soil EC (0.5 dS m^{-1}). However, at 90% WFPS this relationship was mostly

Table 3. Final soil NO_3–N, NH_4–N contents, and redox potential of the soils treated with different levels of initial soil electrical conductivity (EC).

		Final mineral N content†							
		NO_3–N		NH_4–N		Redox potential (90% WFPS)			
Soil	Soil EC	60% WFPS	90% WFPS	60% WFPS	90% WFPS	1 d	3 d	7 d	10 d
	dS m^{-1}	mg N kg^{-1}				mV			
Lin-CS	0.5	2.33*ab*	0.13*a*	1.17*b*	5.37*a*	197	168	37	7
	1.0	2.43*a*	0.14*a*	0.94*c*	4.32*b*	191	183	28	6
	1.5	2.34*ab*	0.15*a*	1.02*bc*	4.20*b*	201	181	39	6
	2.0	2.29*b*	0.13*a*	1.62*a*	4.27*b*	201	175	30	5
Lin-CC	0.5	2.47*a*	0.11*b*	0.93*a*	3.85*a*	204	177	34	6
	1.0	2.36*ab*	0.13*ab*	0.89*a*	3.28*b*	202	180	40	6
	1.5	2.31*b*	0.15*a*	1.30*a*	2.73*c*	201	169	40	6
	2.0	2.27*b*	0.15*a*	1.11*a*	2.80*bc*	178	103	54	9
Mead-CS	0.5	2.09*a*	0.13*a*	1.70*d*	4.20*a*	197	171	64	10
	1.0	1.60*b*	0.16*a*	4.08*c*	4.29*a*	192	167	61	9
	1.5	0.94*c*	0.15*a*	7.22*b*	4.59*a*	195	181	65	8
	2.0	0.70*d*	0.16*a*	7.99*a*	4.84*a*	200	186	59	9
Mead-CC	0.5	3.28*a*	0.13*a*	1.04*bc*	5.00*a*	195	185	72	10
	1.0	3.12*b*	0.15*a*	1.05*c*	3.51*b*	192	183	78	12
	1.5	2.66*c*	0.15*a*	3.07*bc*	3.22*b*	193	181	63	7
	2.0	1.66*d*	0.16*a*	7.73*a*	3.70*b*	189	180	52	8
Man-CC	0.5	2.03*a*	0.06*a*	32.20*b*	31.90*a*	143	113	53	9
	1.0	1.08*b*	0.06*a*	33.20*b*	30.30*ab*	146	120	44	8
	1.5	0.83*c*	0.08*a*	34.80*a*	29.70*bc*	143	118	37	6
	2.0	0.75*d*	0.08*a*	35.60*a*	28.00*c*	145	112	33	2

† Within each column and soil, final mineral N contents followed by the same letter are not significantly different at $P < 0.05$.

Table 4. Averages and standard errors of cumulative N_2O and CO_2 production at different levels of initial soil electrical conductivity (EC) and water-filled pore space (WFPS) during a 10-d incubation at 25°C.

	EC 10 d		N_2O†		CO_2†	
EC 0 d	60%	90%	60% WFPS	90% WFPS	60% WFPS	90% WFPS
	dS m^{-1}		mg N_2O-N m^{-2}		g CO_2–C m^{-2}	
0.5	0.52	0.54	2.00 ± 0.03*a*	3.77 ± 1.10*d*	5.06 ± 0.21*a*	4.39 ± 0.13*a*
1.0	0.99	0.88	1.47 ± 0.02*b*	7.95 ± 0.23*c*	4.31 ± 0.20*b*	3.75 ± 0.13*b*
1.5	1.34	1.29	1.16 ± 0.01*c*	21.90 ± 0.72*b*	3.93 ± 0.19c	3.34 ± 0.17c*b*
2.0	1.77	1.68	0.86 ± 0.01*c*	34.30 ± 1.30*a*	3.66 ± 0.20*c*	3.06 ± 0.11*c*

† Within each column, average gas productions followed by the same letter are not significantly different at $P < 0.05$.

reversed, with four soils showing the highest final NH_4–N at the low EC level (Table 3). The NO_3–N/NH_4–N ratio measured in all soils after incubation varied between water contents and EC levels (Fig. 1). At 60% WFPS, high NO_3–N/NH_4–N ratios occurred in soils with low EC, except for the Man-CC soil that was rich in NH_4–N. At 90% WFPS, however, the NO_3–N/NH_4–N ratio tended to increase with increasing salt addition (Fig. 1).

Nitrous Oxide and Carbon Dioxide Fluxes at 60% Water-Filled Pore Space

Cumulative N_2O emissions during 10-d incubation at 60% WFPS were equivalent to 2.6 to 39.6% of the initial soil NO_3 content. The addition of salt mixtures (EC levels) significantly affected the microbial production of N_2O at 60% WFPS ($P < 0.0001$). In all soils, total N_2O emission during 10-d incubation at 60% WFPS was highest (1.43 to 2.48 mg N_2O-N m^{-2}) when soil EC values were lowest (Fig. 2). Average cumulative N_2O production at 60% WFPS and an initial EC of 0.5 dS m^{-1} was 2.00 mg N_2O-N m^{-2}, whereas 0.86 mg N_2O-N m^{-2} was produced from soils with an initial EC value of 2.0 dS m^{-1} (Table 4). In the Lin-CC soil, for example, estimated average daily N_2O flux rates at 60% WFPS were highest at initial EC of 0.5 dS m^{-1} and lowest at an initial EC of 2.0 dS m^{-1} throughout the entire incubation period (Fig. 3). After a temporary drop in the estimated daily N_2O flux rate during the first day of incubation, the flux rates increased in Lin-CC soil after a 3-d incubation and generally decrease thereafter, presumably due to limited substrate supply (Fig. 3).

Cumulative soil CO_2 emission at 60% WFPS decreased significantly ($P < 0.001$) with increasing initial soil EC level in all soils (Fig. 4). Average soil respiration loss was 5.06 g CO_2–C m^{-2} at 0.5 dS m^{-2} initial soil EC and decreased to 3.66 g CO_2–C m^{-2} when soil EC was raised to 2.0 dS m^{-2} (Table 4), suggesting a general decrease in microbial respiration with increasing EC. Daily CO_2 flux rates varied during the course of incubation and differences among the different EC levels were most pronounced at higher soil respiration rates, e.g., after 1, 3, and 10 d in Lin-CC (Fig. 3). The highest respiration losses were measured in the Man-CC soil (Fig. 4), which also had the highest soil organic C content (Table 1).

Nitrous Oxide and Carbon Dioxide Fluxes at 90% Water-Filled Pore Space

Unlike at 60% WFPS, increasing soil EC significantly increased the production of N_2O ($P < 0.0001$) at 90% WFPS in all soils and this N_2O flux and EC interaction appeared to be nonlinear (Table 4). On average, increasing soil EC from 0.5 to 1.0 dS m^{-1} increased N_2O emission from soil by about 4.2 mg N_2O-N m^{-2}, whereas increasing EC from 1.5 to 2.0 dS m^{-1} caused a 12.4 mg N_2O-N m^{-2} increase in N_2O emission. Depending on the initial EC levels, average total emission of N_2O was 2- to 40-fold higher at 90% WFPS than at 60% WFPS (Table 4, Fig. 3 and 5). With the exception of the Man-CC soil, most of the N_2O release at 90% WFPS occurred within the first 1 to 2 d (Fig. 5). The large pulse of N_2O emission during the first day of incubation was equivalent to 5 to 7% of the initial soil NO_3–N content, indi-

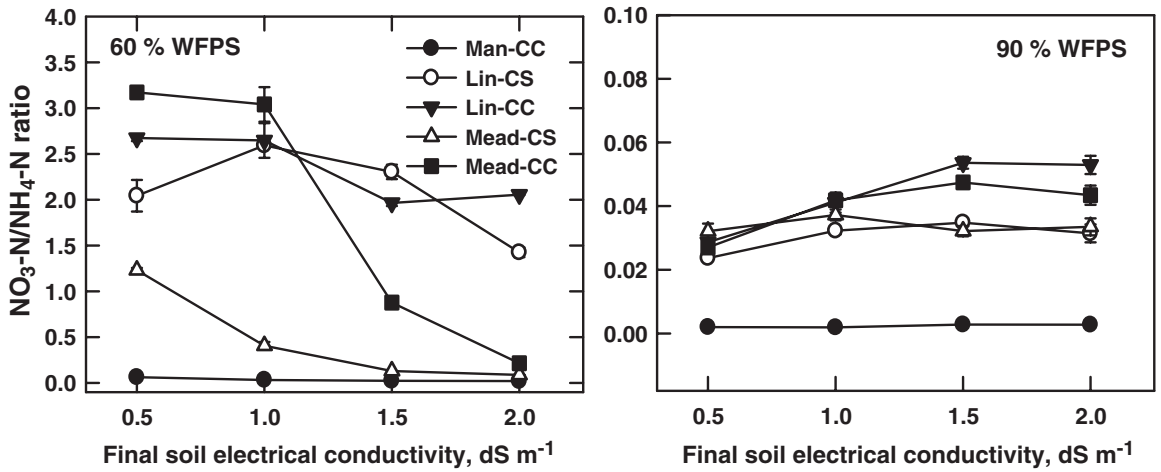

Fig. 1. Ratio of soil NO_3–N and NH_4–N contents in all soils at different levels of soil EC and water contents after a 10-d incubation at 25°C.

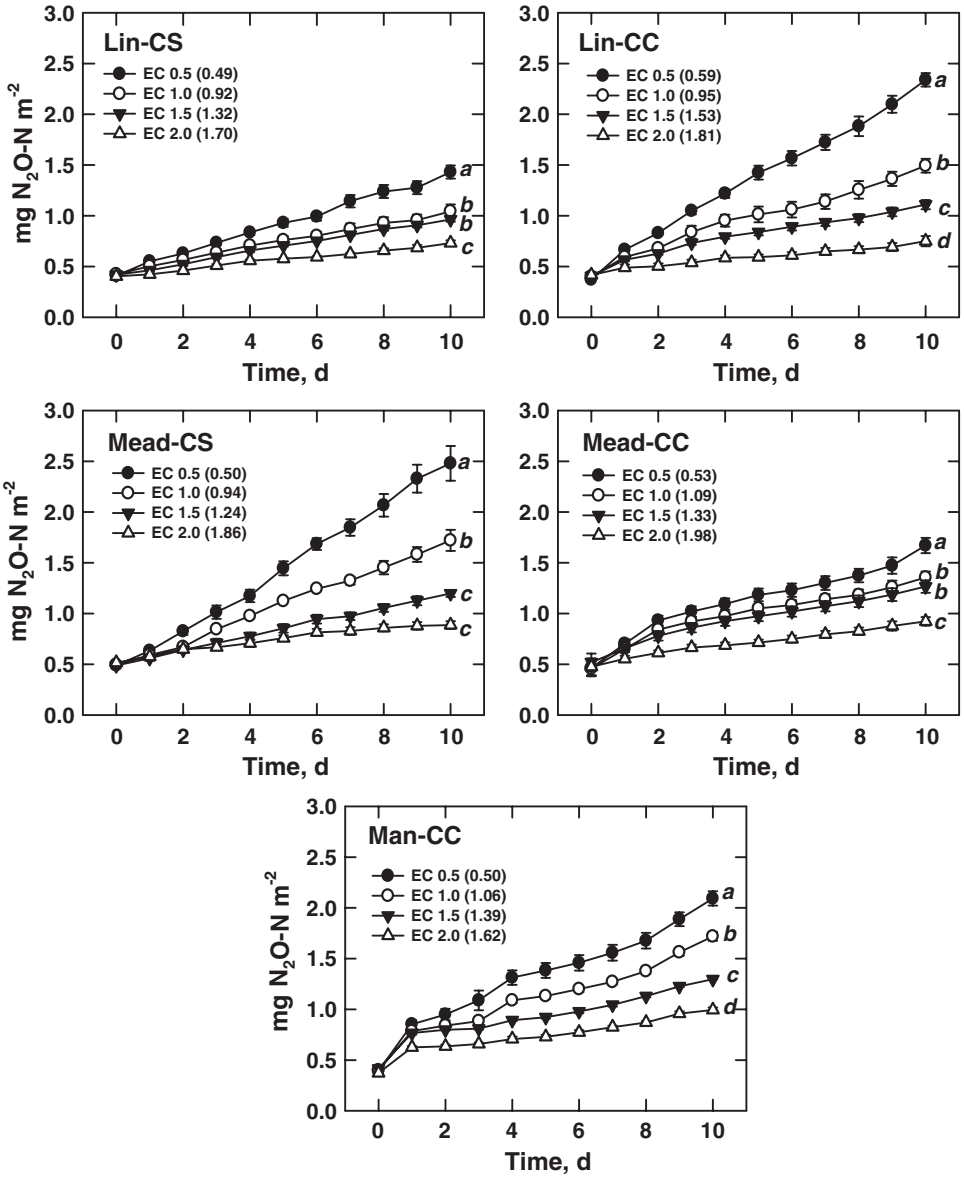

Fig. 2. Cumulative N_2O emissions in all soils at 60% water-filled pore space (WFPS) and four levels of soil electrical conductivity (EC). Vertical bars show standard errors. Values in parenthesis represent the final soil EC (dS m^{-1}) measured after a 10-d incubation at 25°C. Letters at the end of each line indicate significance of differences by LSD test at $\alpha = 0.05$.

cating that despite relatively low initial soil NO_3 content, enough NO_3 was present to attain the high N_2O emission rates measured under depletion of soil oxygen. Depending on the soil and EC level, the proportion of total N_2O produced during the 10-d incubation ranged from 5 to 178% of the initial soil NO_3–N content. This suggests nitrification of available soil NH_4–N to NO_3–N in some soil and EC combinations followed by subsequent denitrification of the NO_3 produced, probably within aerobic or anaerobic microenvironments during the initial incubation stages. Measured redox potentials in the bulk soils remained above 103 mV during the first 3 d of incubation (Table 3), but these bulk soil values probably do not reflect the more heterogeneous mix of aerobic and anaerobic microzones in which nitrification and denitrification may occur more or less simultaneously (Arah, 1990). It is likely that these processes caused the greatly increased N_2O losses measured at 90% WFPS as compared to 60% WFPS. In all soil EC treatments, daily N_2O flux rates decreased over time (see Lin-CC as an example, Fig. 4), suggesting decreasing substrate availability.

Highest cumulative N_2O production and daily initial flux rates generally coincided with high initial soil NO_3–N content. Nitrous oxide flux rates were significantly higher in Mead-CC and Lin-CC soils, probably because nitrification and denitrification rates were higher in the intensively managed continuous maize system than in the maize–soybean rotation with less N input (Table 2). Moreover, the Lin-CC and Mead-CC soils had a history of higher crop residue input than Lin-CS and Mead-CS soils, providing greater C substrate supply to the microbial communities and probably contributing to increased

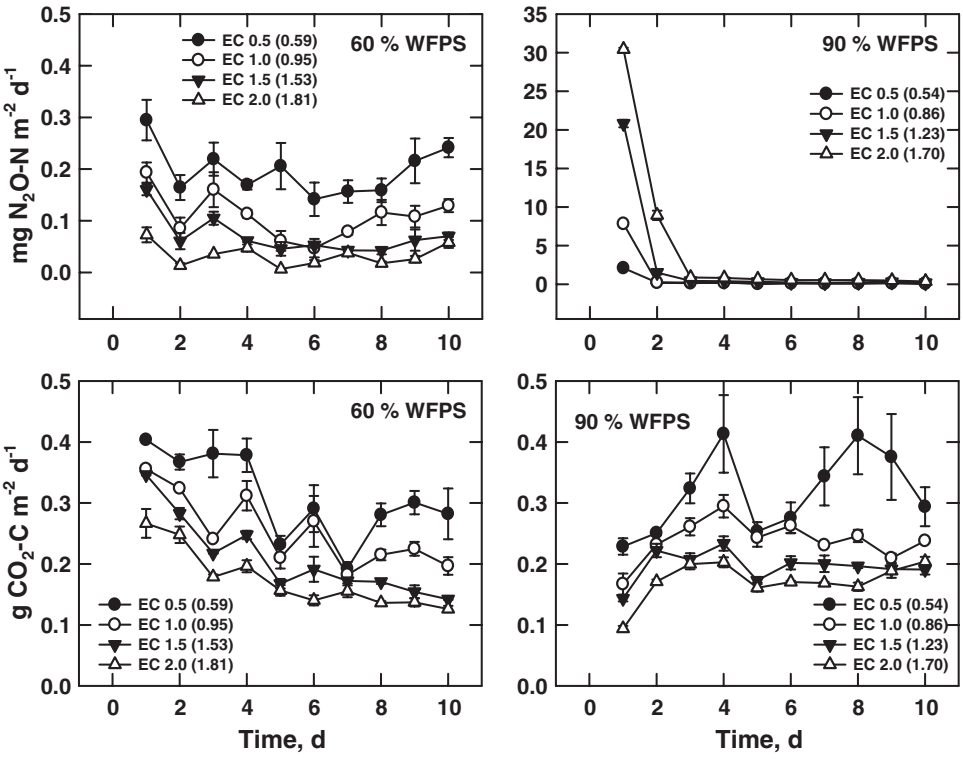

Fig. 3. Measured daily N_2O and CO_2 flux rates in Lin-CC soil at 60 and 90% water-filled pore space (WFPS) and four levels of soil electrical conductivity (EC). Vertical bars show standard errors. Values inside parenthesis show the final soil EC (dS m^{-1}) measured after a 10-d incubation at 25°C.

denitrification. For the Man-CC soil with very low initial soil nitrate content, WFPS and soil EC levels had no effect on N_2O flux after 1 d at 90% WFPS (Fig. 5).

Average cumulative CO_2 production was similar to that measured at 60% WFPS and it also decreased from a high of 4.4 g CO_2–C m^{-2} at 0.5 dS m^{-2} soil EC to a low of 3.1 g CO_2–C m^{-2} when soil EC was raised to 2.0 dS m^{-2} (Table 4, Fig. 6). Daily CO_2 flux rates increased after 1 d and mostly peaked around 4 d of incubation (Fig. 4). There was a decrease in the respiration rate after an 8 d incubation in all treatments and soils, presumably due to exhaustion of C substrates. Similar to 60% WFPS, highest CO_2 losses were measured in the Man-CC soil.

DISCUSSION

Initial soil NO_3–N contents (0.3 to 1.8 mg NO_3–N kg^{-1}, Table 1) in our study were below the levels (3.5 to 10 mg NO_3–N kg^{-1} dry soil) at which significant N_2O fluxes are often reported to occur in the field (Conen et al., 2000; Dobbie et al., 1999; Sehy et al., 2003). Any increase in the substrate N source for N_2O emissions measured during the 10-d incubation was probably the result of N being mineralized and then nitrified by the heterotrophic community. This assumption was supported by the relationship of soil NO_3–N/NH_4–N content ratio to both water contents at increasing EC levels and measured initial and final NO_3–N and NH_4–N contents of the soils (Fig. 1 and Table 3).

At 60% WFPS, the short-term decrease in N_2O flux after a 1-d incubation in all soil EC treatments and soils suggests that low initial soil NO_3–N and low nitrification rates limited microbial N_2O production. As more nitrate was produced over time by nitrification, N_2O flux rates started to increase after a 3-d incubation. The increase in daily respiration rate after a 1-d incubation also supports the assumption that mineralization of organic matter followed by nitrification was the major process that led to production of N_2O at 60% WFPS. At 90% WFPS, N_2O production is primarily related to changes over time in the rates of nitrification, denitrification, NO_3, and carbon substrate supply. Similar to our incubation study, increased N_2O emissions at high WFPS have been observed in many field studies (Abbasi and Adams, 2000; Qian et al., 1997; Sehy et al., 2003; Simojoki and Jaakkola, 2000). Although the measured soil redox potential indicated that at 90% WFPS conditions for denitrification started to prevail in bulk soil within a few days (Table 3), there were probably sufficient microsites to sustain simultaneously high rates of both nitrification and denitrification, particularly near the soil surface where O_2 concentration was high, resulting in high flux rates during the first days of incubation. The variation among soils in the patterns of N_2O emission at 90% WFPS (Fig. 5) was probably caused by differences in such microsites and in substrate supply among the experimental soils, including the differences in the NO_3 supply relative to the available C substrate (Weier et al., 1993).

Our study showed that the N_2O and CO_2 production by the microbial community were affected by salt concentration (in situ soil EC) at both water contents and even at low soil nitrate content. Increasing soil EC decreased heterotrophic soil respiration at 60 and 90% WFPS, sug-

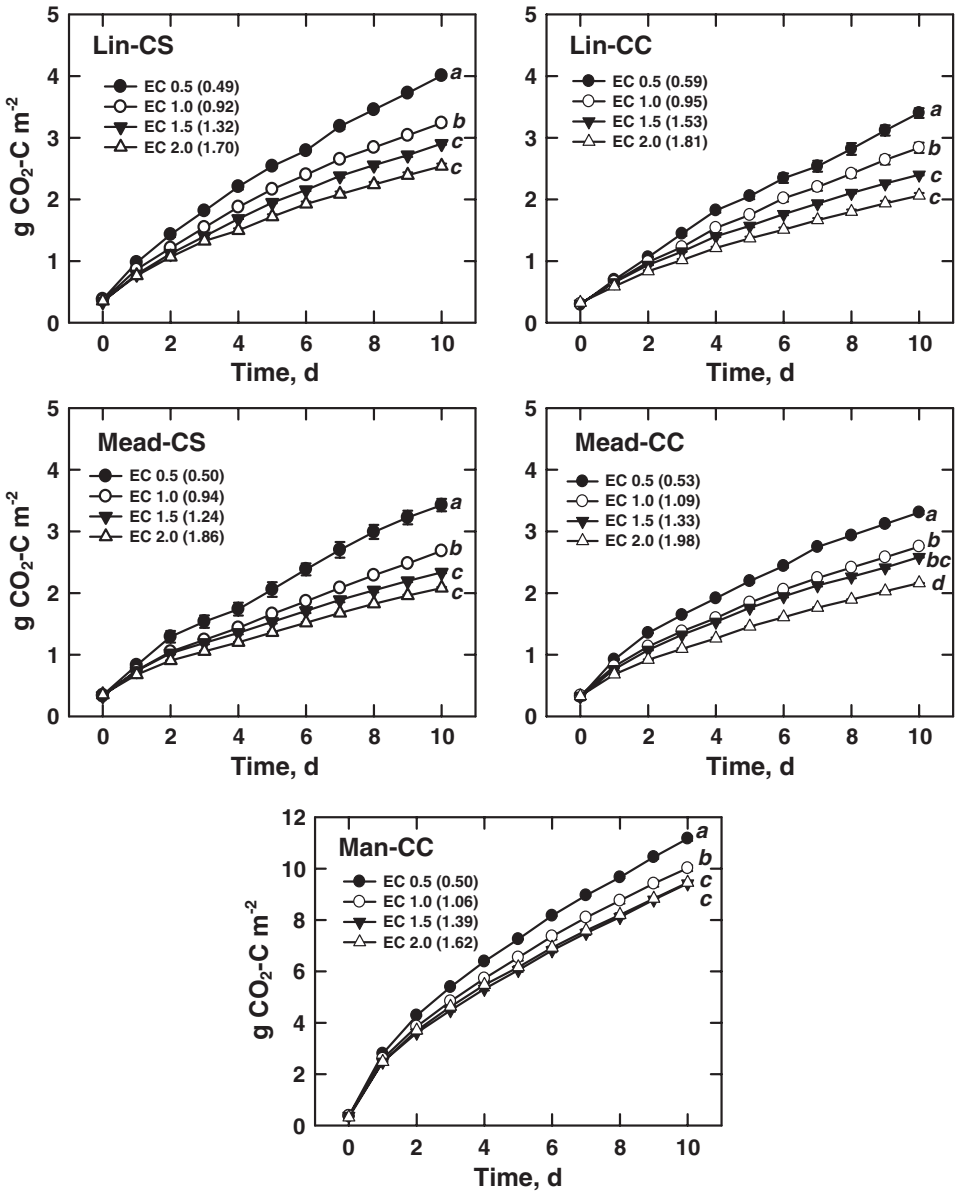

Fig. 4. Cumulative CO_2 emissions in all soils at 60% water-filled pore space (WFPS) and four levels of soil electrical conductivity (EC). Vertical bars show standard errors. Values in parenthesis represent the final soil EC (dS m^{-1}) measured after a 10-d incubation at 25°C. Letters at the end of each line indicate significance of differences by LSD test at $\alpha = 0.05$.

gesting a general reduction in microbial activity due to increased osmotic stress on the microbial communities. Killham (1985) also found a decrease in respired C and dehydrogenase activity when a sandy loam soil was irrigated with increasing salinity. Frankenberger and Bingham (1982) reported that dehydrogenase activity was severely inhibited (30 to 81%) when the EC of a soil extract (EC_e) was increased from 0.2 to 22 dS m^{-1}. Soil CO_2 production at various EC levels was positively correlated with soil organic C content ($P < 0.01$), which has also been found in other studies (Zaman et al., 2004).

The observed interactions between increasing salt concentrations, water content, and N_2O emissions from soils are likely to be related to the physiological status of microbes involved in nitrification and denitrification. Largest NH_4–N concentration was generally observed at 60% WFPS and an EC of 2.0 dS m^{-1}, whereas it was lowest in soils with low EC (Table 3). This suggests that, unlike C mineralization (Fig. 4), N mineralization was not suppressed by increasing EC, whereas nitrification of ammonium to nitrate was inhibited by increasing salt concentrations. Similar observations have been made in a number of studies with widely varying EC levels, including saline and alkaline soils (Laura, 1974; McClung and Frankenberger, 1985; McCormick and Wolf, 1980; Pathak and Rao, 1998). Added salt mixtures increase the concentration of ions in the soil solution and decrease available water for microbial activity. To survive under stress, soil microbial communities change their microbial functions and/or activity in response to environmental disturbances. Several reports suggest that bacteria involved in nitrification regulate intracellular solute concentrations through

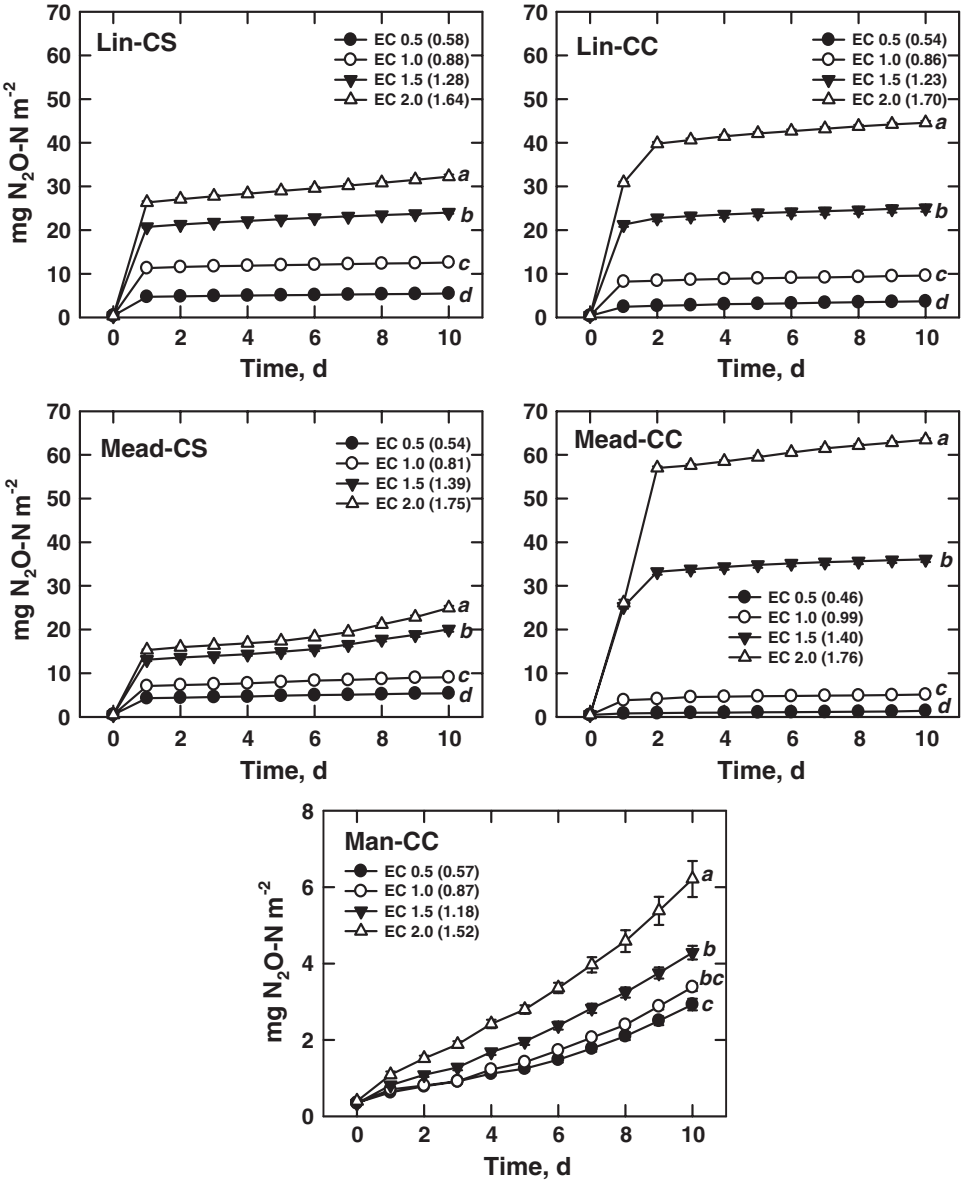

Fig. 5. Cumulative N_2O emissions in all soils at 90% water-filled pore space (WFPS) and four levels of soil electrical conductivity (EC). Vertical bars show standard errors. Values in parenthesis represent the final soil EC (dS m^{-1}) measured after a 10-d incubation at 25°C. Letters at the end of each line indicate significance of differences by LSD test at $\alpha = 0.05$.

accumulation and loss of amino acids (Schall et al., 1981; Killham and Firestone, 1984). More generally, the ability to do this may vary among microbial communities. Studies by Stark and Firestone (1995) indicated that there was a 75% decrease in nitrification rates when soil was exposed to 0.67 *M* K_2SO_4 solutions. Microbial nitrification was affected because the bacteria involved were unable to produce compatible solutes as the cell underwent dehydration. In our study, this would explain the general reduction in N_2O emissions with increasing EC at 60% WFPS. Other indications for this include the lack of significant NO_3–N accumulation at EC > 1 dS m^{-1} (Table 3) and slightly decreasing final soil EC values (Table 4).

Microbial cells may also employ another response mechanism to salt stress by catabolizing intracellular solutes to CO_2 or polymerizing them into osmotically less active compounds (Halverson et al., 2000; Avron and Ben-Amotz, 1979; Reed and Stewart, 1983). Considering this, it is possible that denitrifiers may employ several response mechanisms to salt stress that lead to a generally greater tolerance than that of nitrifiers. Halverson et al. (2000) reported that in response to addition of 0.11 *M* NaCl, *Pseudomonas spp*. released a maximum of 22 to 26% of their amino acid pool and 11 to 21% of their low molecular weight in neutral sugars. Korber et al. (1996) reported that *Pseudomonas fluorescens*, a common denitrifying bacteria in soils, plasmolyzed on the addition of 1.0 to 1.5 *M* NaCl, but were able to resume growth after removal of the salt stress. In our study, it appears that at 2.0 dS m^{-1} initial soil EC and 90% WFPS, where the salt concentration was a 0.03 to 0.1 *M* KCl, Na_2SO_3, and $CaCl_2$ mixture, bacterial communities dominated by denitrifiers

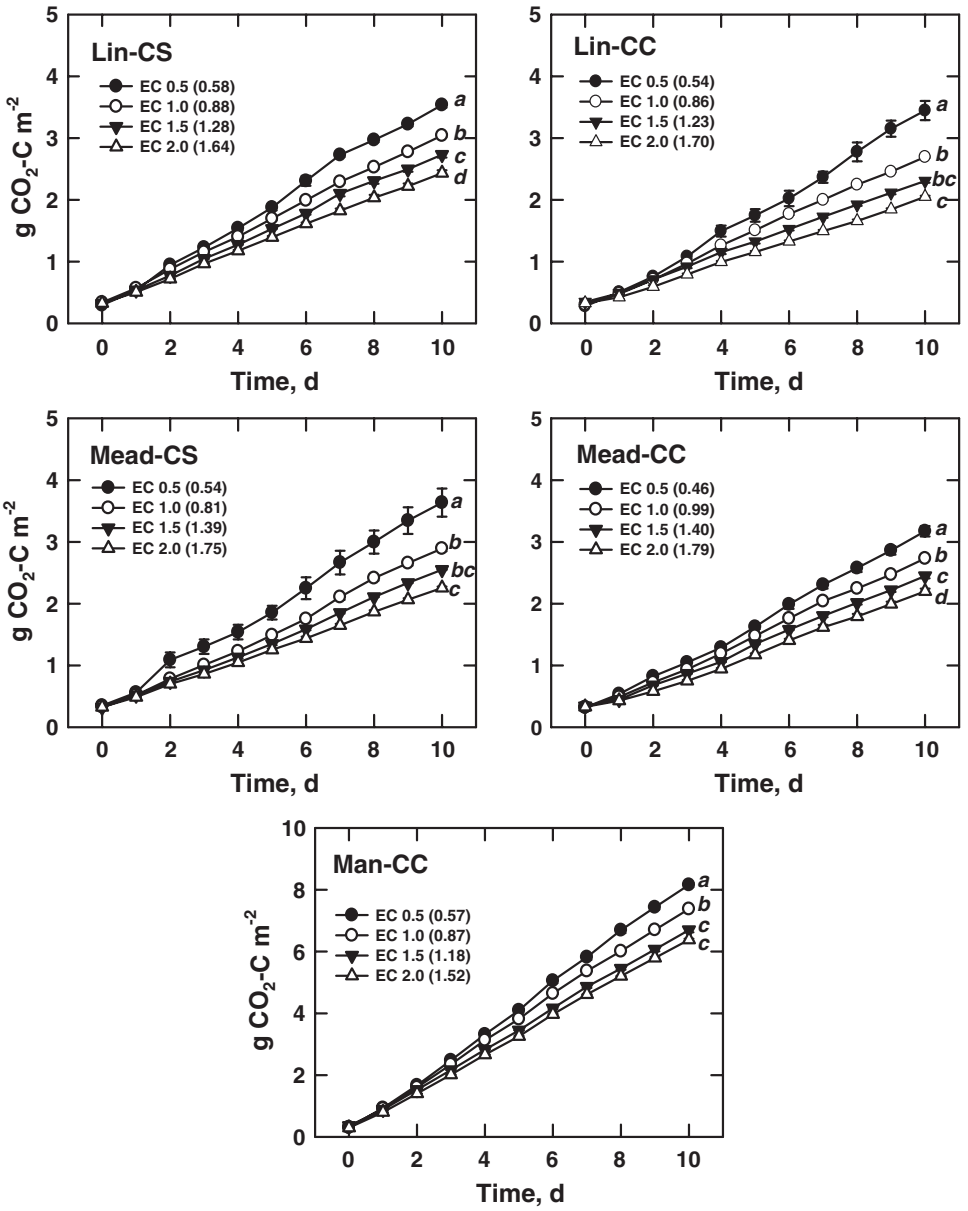

Fig. 6. Cumulative CO_2 emissions in all soils at 90% water-filled pore space (WFPS) and four levels of soil electrical conductivity (EC). Vertical bars show standard errors. Values in parenthesis represent the final soil EC (dS m^{-1}) measured after a 10-d incubation at 25°C. Letters at the end of each line indicate significance of differences by LSD test at $\alpha = 0.05$.

performed their metabolic functions better than nitrifying bacteria at 60% WFPS by diluting the external salt stress through response mechanisms such as those discussed above. Another indirect indication for a more efficient response of denitrifiers to higher salt concentrations is the somewhat greater decline in EC and the overall large loss of soil NO_3 observed in all soils during the 10-d incubation at 90% WFPS.

With the salt additions used in this study, the presence of specific ions may have also caused nutritional imbalances that affected microbial growth rates and enzyme activities. Several studies noted that reduction of nitrification rates was more severe in the presence of chloride salts when compared to the sulfates added at equivalent rates (Frankenberger and Bingham, 1982; Dinesh et al., 1995; McClung and Frankenberger, 1985; Agarwal et al., 1971). They attributed the lower degree of inhibition by sulfates to the enhanced utilization of sulfate anions in microbial cells for the synthesis of amino acids, vitamins, sulfolipids, and several other essential organic compounds. In contrast, chloride ions are less likely to be incorporated in anabolic pathways and hence may accumulate in soils at toxic levels. In our study, because Cl^- and SO_4^{2-} ions were used in the salt mixtures and the concentration of Cl^- ions in each EC levels was relatively high, toxic effects of chloride on soil microbes may have contributed to reduced N_2O production when salt concentration increased from 0.01 (0.5 dS m^{-1}) to 0.48 (2.0 dS m^{-1}) *M* salt mixtures at 60% WFPS. However, at high water content (90% WFPS) significant nitrate production and denitrification occurred at comparable levels of salt addition, indicating that inhibition of nitrification by Cl^-

and SO_4^{2-} ions was probably negligible. The data presented here do not allow a more detailed interpretation of potential selective toxicities of specific ions.

The range of initial bulk soil EC chosen for this study, 0.5 to 2.0 dS m^{-1}, was relatively narrow, but it represents conditions found in many agricultural soils. It is also typical for temporary or localized increases in EC that may result from fertilizer application (Amos et al., 2005). Within that range, effects of EC on N_2O and CO_2 emissions were most significant at initial EC levels of >1 dS m^{-1} and in soils with greater biological activity. Our experiment did not allow for the derivation of robust quantitative relationships between CO_2 or N_2O fluxes and soil EC that would be applicable to field conditions, including the presence of growing plants. More research should be conducted on this topic so that direct effects of soil EC on soil greenhouse gas production can also be accounted for in ecosystem simulation models that aim to predict greenhouse gas emissions from soil. Because we did not measure community structures of ammonia oxidizers and denitrifiers or the physiological or metabolic state of the microorganisms at increasing soil EC levels, the above discussion on mechanisms of soil EC effects on trace gas evolution at different levels of water content requires further verification in the field. In particular, the mechanisms used by soil microorganisms in response to stress (soil EC > 1.0 dS m^{-1}) and the types of microorganisms proliferating during high salt conditions are not well understood.

CONCLUSIONS

In the presence of low soil NO_3–N content, soil EC affects microbially driven production of greenhouse gases, but its direct influence varies according to water content and EC level. Average microbial respiration decreased with increasing the initial soil EC from 0.5 to 2.0 dS m^{-1} regardless of soil water status. At 60% WFPS, N_2O production decreased with increasing EC in all soils. At 90% WFPS, N_2O production was greater than that at 60% WFPS and N_2O losses increased with increasing soil EC. Differences in greenhouse gas emissions due to varying soil EC and WFPS may be related to changes in mechanisms of adjustment to salt stress by microbial communities and microbial toxicities to specific ions. The general decrease in soil respiration with increasing soil EC may have been due to osmotic stress on microbial activities. At 60% WFPS, the reduced N_2O production at increased EC was most likely associated with decreasing activity of nitrifying bacteria, which became stressed at higher EC levels. However, when soil water content was raised to 90% WFPS, denitrification was the most likely primary source of N_2O and the microbial community involved in the process appears to be more tolerant to salt stress. The major implication arising from our laboratory study is that ecosystem models that attempt to simulate N_2O and CO_2 fluxes from soils may have to also account for general or short-term effects of rising or declining soil EC on microbial activities. Whether the relationships shown in the laboratory are of equal importance under field conditions remains to be verified. Likewise, better understanding of physiological shifts in the soil microbial community in response to increasing soil EC or water content is needed.

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